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Cottick

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Paul R. Schimmel

Serial No.: 07/586,534

Art Unit: 1807

Filed: September 21, 1990

Examiner: A. Yarbrough

For: DESIGNING COMPOUNDS SPECIFICALLY INHIBITING
RIBONUCLEIC ACID

Commissioner of Patents and Trademarks
Washington, D.C. 20231

DECLARATION OF PAUL R. SCHIMMEL UNDER 37 C.F.R. §1.132

Sir:

I, Paul R. Schimmel, do hereby declare:

1. I received an A.B. degree from Ohio Wesleyan University in 1962, attended Tufts University School of Medicine from 1962-1963, and received a Ph.D. degree from the Massachusetts Institute of Technology in 1966.

I am a tenured professor of Biochemistry and Biophysics at the Massachusetts Institute of Technology, and have had the honor of being appointed the John D. MacArthur Professor of Biochemistry and Biophysics at the Massachusetts Institute of Technology since 1992.

I have been conducting research in Molecular Biology and Biochemistry for 28 years.

I have authored or co-authored approximately 200 publications and co-authored the text "Biophysical Chemistry", Cantor and Schimmel, W.H. Freeman and Company 1980.

I am a consultant for several companies active in drug design and pharmaceuticals in general.

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I am currently on, or have been on, the editorial boards of the following publications: *Accounts of Chemical Research*, *Archives of Biochemistry and Biophysics*, *Biochemistry*, *Biopolymers*, *International Journal of Biological Macromolecules*, *Journal of Biological Chemistry*, *Nucleic Acids Research*, *Trends in Biochemical Sciences*, *University Books*, *European Journal of Biochemistry* and *Protein Science*.

2. I am an inventor of the claimed method in the above-referenced application. This application discloses a method for inhibiting RNA function by binding a compound to nucleotides exposed on the surface of the minor groove of the RNA molecule. The presence of the compound within the minor groove impairs or prevents replication or other functions of the RNA molecule.

3. I conducted experiments with my colleague, Karin Musier-Forsyth, to study the interactions of a class II tRNA synthetase of *E. coli* with specific regions within the minor groove of tRNA^{Ala}. The results of our experiments were published in the well known scientific journal *Nature* 357:513-515 (1992). A reprint of our paper is attached as Exhibit A.

Previous studies demonstrated that a single base pair (G3:U70) in the acceptor stem of tRNA^{Ala} is important for alanine-tRNA synthetase recognition. The G3 exocyclic 2-amino group projects into the minor groove of the tRNA molecule and interacts with the tRNA synthetase molecule as it couples to the tRNA molecule within the minor groove. Removal of this G3 2-amino group eliminates aminoacylation. The 2-amino group is flanked on either side by ribose 2'-hydroxyl groups lining the minor groove.

As described in the attached paper, we made deoxy and O-methyl substitutions of individual and multiple 2'-hydroxyl groups near and beyond the G3:U70 base pair in order to demonstrate that the 2'-hydroxyl groups lining the minor groove are essential for tRNA recognition and aminoacylation.

The significance of 2'-hydroxyls in mediating an RNA-protein interaction is summarized in the model of the alanine acceptor helix shown in Figure 3 of the paper. Removal of the atoms shown in pale blue had little or no effect on the aminoacylation efficiency of the duplex. Of all the single substitutions examined, incorporation of inosine at position 3 (which removes the 2-amino group shown in red) had the greatest effect on helix recognition by alanine-tRNA synthetase, eliminating detectable aminoacylation. Additional atoms of functional importance identified in this study are shown in white in Figure 3.

Results using 2'-O-methyl-substituted nucleotides support the hypothesis that functionally unimportant 2'-OH groups are not in close contact with the enzyme and raise the possibility that the functionally important 2'-hydroxyls are involved in hydrogen bonding interactions with alanine-tRNA synthetase either directly or through bridging water molecules. The three functional hydroxyl groups (the 2'-hydroxyls of G4, U70 and C71) are clustered within approximately 5 Angstroms of the exocyclic 2-amino group of G3.

Our experiments illustrate that, within the cluster of minor groove interactions flanking the 2-amino group of G3, functional contacts with 2'-OH groups are quantitatively at least as significant as base-specific interactions.

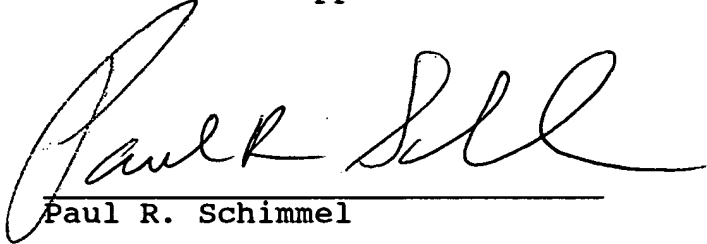
4. In my expert opinion, a person skilled in the art at the time the application was filed would have been able to

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conduct substitution experiments, such as those described in the attached publication, to determine other critical sites within the minor groove of an RNA molecule, such as the ribose 2'-hydroxyl groups described in the paper. Upon learning the exact location of these critical sites, one could routinely synthesize organic compounds or isolate proteins, such as inactive enzymes or portions thereof, that would bind to the critical site within the minor groove and prevent binding of the active enzyme necessary for RNA function.

5. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like are made punishable by fine or imprisonment or both under Sec. 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the above-identified application or any patent issuing thereon.

7/27/92
Date


Paul R. Schimmel